

## AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

### Listing of Claims:

1-115. **(Canceled)**

116. **(Currently amended)** A system for control of gene expression comprising:

(i) an isolated first nucleic acid molecule comprising a cis-repressive sequence element upstream of an open reading frame (ORF), or including part of the open reading frame, wherein the cis-repressive sequence element forms part of a stem-loop structure that represses translation of the ORF; and

(ii) an isolated second nucleic acid molecule comprising first and second stem-forming portions and a non-stem-forming portion, wherein the non-stem-forming portion connects the 3' end of the first stem-forming portion and the 5' end of the second stem-forming portion to form a loop, and wherein a portion ~~of at least 6 nucleotides in length~~ of the first or second stem-forming portion of the second nucleic acid molecule ~~that is at least 6 nucleotides in length~~ is complementary or substantially complementary to a portion ~~of at least 6 nucleotides in length~~ of the first nucleic acid molecule ~~that is at least 6 nucleotides in length~~ and interacts with the first nucleic acid molecule to disrupt the stem-loop structure in which the cis-repressive sequence element participates and thereby derepress translation of the ORF.

117-176. **(Canceled)**

177. **(Withdrawn)** A kit for allowing a user to regulate expression of a gene of choice comprising:

(a) a first plasmid comprising

(i) a template for transcription of a cis-repressive RNA element; and

(ii) a promoter located upstream of the template for transcription of the cis-repressive RNA element;

(b) a second plasmid comprising

(i) a template for transcription of a cognate trans-activating RNA element; and

(ii) a promoter located upstream of the template for transcription of the trans-activating RNA element; and

(c) one or more elements selected from the list consisting of: (i) one or more inducers; (ii) host cells; (iii) one or more buffers; (iv) an enzyme, e.g., a restriction enzyme; (v) DNA isolation reagents; (vi) DNA purification reagents; (vii) a control plasmid lacking a crRNA or taRNA sequence; (viii) a control plasmid containing a crRNA or taRNA sequence or both; (ix) sequencing primers; and (x) instructions for use.

178. **(Withdrawn)** A kit for allowing a user to regulate expression of a gene of choice comprising:

a plasmid comprising a template for transcription of a cis-repressive RNA element and a promoter located upstream of the template for transcription of the cis-repressive RNA element and further comprising a template for transcription of a cognate trans-activating RNA element and a promoter located upstream of the template for transcription of the cognate trans-activating RNA element; and

one or more elements selected from the list consisting of: (i) one or more inducers; (ii) host cells; (iii) one or more buffers; (iv) an enzyme, e.g., a restriction enzyme; (v) DNA isolation reagents; (vi) DNA purification reagents; (vii) a control plasmid lacking a crRNA or taRNA sequence; (viii) a control plasmid containing a crRNA or taRNA sequence or both; (ix) sequencing primers; and (x) instructions for use.

179. **(Withdrawn)** A kit for allowing a user to regulate expression of a gene of choice comprising:

(a) a first plasmid comprising

- (i) a template for transcription of a cis-repressive RNA element; and
- (ii) a promoter located upstream of the template for transcription of the cis-repressive RNA element;

(b) a second plasmid comprising

- (i) a template for transcription of a cognate trans-activating RNA element; and
- (ii) a promoter located upstream of the template for transcription of the trans-activating RNA element;

(c) a third plasmid comprising a template for transcription of a cis-repressive RNA element and a promoter located upstream of the template for transcription of the cis-repressive RNA element and further comprising a template for transcription of a cognate trans-activating RNA element and a promoter located upstream of the template for transcription of the cognate trans-activating RNA element; and

(d) one or more elements selected from the list consisting of: (i) one or more inducers; (ii) host cells; (iii) one or more buffers; (iv) an enzyme, e.g., a restriction enzyme; (v) DNA isolation reagents; (vi) DNA purification reagents; (vii) a control plasmid lacking a crRNA or taRNA sequence; (viii) a control plasmid containing a crRNA or taRNA sequence or both; (ix) sequencing primers; and (x) instructions for use.

180. **(Previously presented)** A kit comprising:

one or more oligonucleotides comprising a crRNA sequence, one or more oligonucleotides comprising a taRNA sequence, or one or more oligonucleotides comprising a crRNA sequence and one or more oligonucleotides comprising a taRNA sequence, wherein the kit further comprises one or more items selected from the group consisting of: (i) one or more inducers; (ii) host cells; (iii) one or more buffers; (iv) an enzyme, e.g., a restriction enzyme; (v) DNA isolation reagents; (vi) DNA purification reagents; (vii) a control plasmid lacking a crRNA or taRNA sequence; (viii) a control

plasmid containing a crRNA or taRNA sequence or both; (ix) sequencing primers; and (x) instructions for use.

181. **(Withdrawn)** A method of regulating translation of an open reading frame comprising steps of:

introducing an engineered template for transcription of an mRNA into a cell and allowing mRNA transcription to occur resulting in a transcribed mRNA, wherein the template is engineered so that the transcribed mRNA comprises first and second nucleic acid elements that form a stem-loop structure that represses translation of the mRNA; and providing an engineered nucleic acid molecule that interacts with the mRNA so as to derepress translation of the mRNA to the cell.

182. **(Withdrawn)** The method of claim 181, wherein the engineered template comprises:

(i) a first stem-forming portion;  
(ii) a second stem-forming portion, wherein the two stem-forming portions are complementary or substantially complementary;  
(iii) a non-stem-forming portion connecting the 3' end of the first stem-forming portion and the 5' end of the second stem-forming portion; and  
(iv) an open reading frame (ORF),

wherein the engineered nucleic acid molecule forms a stem-loop structure that represses translation of the ORF.

183-242. **(Canceled)**

243. **(Withdrawn)** The method of claim 181, wherein the engineered nucleic acid molecule comprises:

(i) a first stem-forming portion;  
(ii) a second stem-forming portion; and  
(iii) a non-stem-forming portion, wherein the non-stem-forming portion connects the 3' end of the first stem forming portion and the 5' end of the second stem-forming portion to form a loop,

and wherein a portion of the nucleic acid molecule is complementary or substantially complementary, to a portion of the transcribed mRNA.

244. **(Previously presented)** The system of claim 116, wherein the first nucleic acid molecule represses translation by at least 80%.
245. **(Previously presented)** The system of claim 116, wherein the first nucleic acid molecule represses translation by at least 90%.
246. **(Previously presented)** The system of claim 116, wherein the first nucleic acid molecule represses translation by at least 98%.
247. **(Previously presented)** The system of claim 116, wherein the second nucleic acid molecule activates translation by at least 5 fold.
248. **(Previously presented)** The system of claim 116, wherein the second nucleic acid molecule activates translation by at least 10 fold.
249. **(Previously presented)** The system of claim 116, wherein the second nucleic acid molecule activates translation by at least 19 fold.
250. **(Previously presented)** The system of claim 116, wherein the first and second nucleic acid molecules are composed of RNA.
251. **(Withdrawn)** The system of claim 116, wherein the first and second nucleic acid molecules are composed of DNA.
252. **(Withdrawn)** The system of claim 116, wherein the first and second nucleic acid molecules are composed of DNA and RNA.
253. **(Previously presented)** The system of claim 116, wherein the cis-repressive sequence element is positioned upstream of the ORF.

254. **(Previously presented)** The system of claim 116, wherein the first nucleic acid molecule comprises:

- (i) a first stem-forming portion;
- (ii) a second stem-forming portion, wherein the two stem-forming portions are complementary or substantially complementary and at least 4 nucleotides in length, and
- (iii) a non-stem-forming portion that forms a loop connecting the 3' end of the first stem-forming portion and the 5' end of the second stem-forming portion, wherein the two stem-forming portions and the non-stem forming portion of the first nucleic acid molecule form the stem-loop structure that represses translation of the ORF.

255. **(Previously presented)** The system of claim 254, wherein the first and second stem-forming portions of the first nucleic acid molecule are substantially complementary.

256. **(Currently amended)** The system of claim 116, wherein a portion of the cis-repressive sequence element that is of at least 4 nucleotides in length is complementary or substantially complementary to a ribosome binding site (RBS) sequence.

257. **(Currently amended)** The system of claim 116, wherein a portion of the cis-repressive sequence element that is of at least 4 nucleotides in length is complementary or substantially complementary to a Kozak consensus sequence.

258. **(Previously presented)** The system of claim 254, wherein the second stem-forming portion of the first nucleic acid molecule comprises all or part of an RBS sequence.

259. **(Previously presented)** The system of claim 254, wherein the non-stem-forming portion of the first nucleic acid molecule comprises a YUNR sequence.

260. **(Previously presented)** The system of claim 254, wherein the non-stem-forming portion of the first nucleic acid molecule is 4, 5, 6, 7, 8, 9, 10, 11, or 12 nucleotides in length.

261. **(Withdrawn)** The system of claim 254, wherein the non-stem-forming portion is between 13 and 50 nucleotides in length, inclusive.

262. **(Previously presented)** The system of claim 254, wherein the length of the stem formed by the two stem-forming portions of the first nucleic acid molecule is between 4 and 100 nucleotides, inclusive.

263. **(Previously presented)** The system of claim 254, wherein the length of the stem formed by the two stem-forming portions of the first nucleic acid molecule is between 6 and 50 nucleotides, inclusive.

264. **(Previously presented)** The system of claim 254, wherein the length of the stem formed by the two stem-forming portions of the first nucleic acid molecule is between 12 and 30 nucleotides, inclusive.

265. **(Previously presented)** The system of claim 254, wherein the length of the stem formed by the two stem-forming portions of the first nucleic acid molecule is approximately 19 nucleotides.

266. **(Previously presented)** The system of claim 254, wherein the two stem-forming portions of the first nucleic acid molecule exhibit at least 66% complementarity.

267. **(Previously presented)** The system of claim 254, wherein the two stem-forming portions of the first nucleic acid molecule exhibit between 75 and 95% complementarity.

268. **(Previously presented)** The system of claim 254, wherein the two stem-forming portions of the first nucleic acid molecule exhibit approximately 85% complementarity.

269. **(Previously presented)** The system of claim 254, wherein the stem formed by the two stem-forming portions of the first nucleic acid molecule includes at least one area of non-complementarity.

270. **(Previously presented)** The system of claim 269, wherein the stem formed by the two stem-forming portions of the first nucleic acid molecule includes at least one bulge.
271. **(Previously presented)** The system of claim 254, wherein the stem formed by the two stem-forming portions of the first nucleic acid molecule includes at least two dispersed areas of non-complementarity.
272. **(Previously presented)** The system of claim 271, wherein the stem formed by the two stem-forming portions of the first nucleic acid molecule includes at least two dispersed bulges.
273. **(Previously presented)** The system of claim 254, wherein the stem formed by the two stem-forming portions of the first nucleic acid molecule includes at least three dispersed areas of non-complementarity.
274. **(Previously presented)** The system of claim 273, wherein the stem formed by the two stem-forming portions of the first nucleic acid molecule includes at least three dispersed bulges.
275. **(Previously presented)** The system of claim 116, wherein the first nucleic acid molecule forms a single stable stem.
276. **(Previously presented)** The system of claim 116, wherein the first nucleic acid molecule represses translation in the absence of a ligand.
277. **(Previously presented)** The system of claim 254, wherein the second stem-forming portion of the first nucleic acid molecule comprises a 5' portion of the ORF.
278. **(Previously presented)** The system of claim 254, wherein the first nucleic acid molecule comprises a start codon.

279. **(Previously presented)** The system of claim 278, wherein the first nucleic acid molecule comprises a spacer comprising one or more nucleotides between the 3' end of the second stem-forming portion and the start codon.

280. **(Withdrawn)** The system of claim 278, wherein all or part of the start codon is located within the second stem-forming portion.

281. **(Previously presented)** The system of claim 254, wherein the first nucleic acid molecule comprises one or more nucleotides at the 5' end that do not participate in the stem-loop structure.

282. **(Previously presented)** The system of claim 254, wherein the first nucleic acid molecule comprises between 5 and 50 nucleotides upstream of the 5' end of the first stem-forming portion.

283. **(Previously presented)** The system of claim 116, wherein the first nucleic acid molecule comprises a ligand binding domain.

284. **(Currently amended)** The system of claim 254, wherein the first nucleic acid molecule comprises a third stem-forming portion that is of at least 4 nucleotides in length that is complementary or substantially complementary to the second stem-forming portion, wherein the first and third stem-forming portions form alternate stem-loop structures with the second stem-forming portion.

285. **(Previously presented)** The system of claim 284, wherein the second stem-forming portion of the first nucleic acid molecule comprises all or part of an RBS sequence.

286. **(Previously presented)** The system of claim 116, wherein the second nucleic acid molecule comprises a YNAR sequence positioned 5' to the 5' end of the first stem-forming portion.

287. **(Previously presented)** The system of claim 116, wherein the length of the stem formed by the two stem-forming portions of the second nucleic acid molecule is between 6 and 50 nucleotides.

288. **(Previously presented)** The system of claim 116, wherein the length of the stem formed by the two stem-forming portions of the second nucleic acid molecule is between 12 and 30 nucleotides.

289. **(Withdrawn)** The system of claim 116, wherein the length of the stem formed by the two stem-forming portions of the second nucleic acid molecule is approximately 19 nucleotides.

290. **(Previously presented)** The system of claim 116, wherein the two stem-forming portions of the second nucleic acid molecule exhibit at least 66% complementarity.

291. **(Previously presented)** The system of claim 116, wherein the two stem-forming portions of the second nucleic acid molecule exhibit between 75 and 95% complementarity.

292. **(Withdrawn)** The system of claim 116, wherein the two stem-forming portions of the second nucleic acid molecule exhibit approximately 85% complementarity.

293. **(Previously presented)** The system of claim 116, wherein the stem formed by the two stem-forming portions of the second nucleic acid molecule includes at least one area of non-complementarity.

294. **(Previously presented)** The system of claim 116, wherein the stem formed by the two stem-forming portions of the second nucleic acid molecule includes at least two dispersed areas of non-complementarity.

295. **(Withdrawn)** The system of claim 116, wherein the stem formed by the two stem-forming portions of the second nucleic acid molecule includes at least three dispersed areas of non-complementarity.

296. **(Previously presented)** The system of claim 116, wherein the second nucleic acid molecule comprises a nucleotide analog.

297. **(Previously presented)** The system of claim 116, wherein the second nucleic acid molecule comprises a ligand binding domain.

298. **(Canceled)**

299. **(Withdrawn)** The system of claim 116, wherein the first nucleic acid molecule has the sequence of crR10 and the second nucleic acid molecule has the sequence of taR10.

300. **(Previously presented)** The system of claim 116, wherein the first nucleic acid molecule has the sequence of crR12 (SEQ ID NO:56) and the second nucleic acid molecule has the sequence of taR12 (SEQ ID NO:55).

301. **(Withdrawn)** The system of claim 116, wherein the first nucleic acid molecule has the sequence of crR10 or a variant of crR10 that differs from crR10 by 12 nucleotides or less and includes at least 3 dispersed areas of non-complementarity and the second nucleic acid molecule has the sequence of taR10 or a variant of taR10 that differs from taR10 by 12 nucleotides or less and includes at least 3 dispersed areas of non-complementarity.

302. **(Previously presented)** The system of claim 116, wherein the first nucleic acid molecule has the sequence of crR12 (SEQ ID NO:56) or a variant of crR12 (SEQ ID NO:56) that differs from crR12 (SEQ ID NO:56) by 12 nucleotides or less and includes at least 3 dispersed areas of non-complementarity and the second nucleic acid molecule has the sequence of taR12 (SEQ ID NO:55) or a variant of taR12 (SEQ ID NO:55) that differs from taR12 (SEQ ID NO:55) by 12 nucleotides or less and includes at least 3 dispersed areas of non-complementarity.

303. **(Previously presented)** The system of claim 116, wherein the first nucleic acid molecule and the second nucleic acid molecule have an equilibrium association constant between  $0.8 \times 10^7$  and  $1.5 \times 10^7$  kcal/mol.